Remarks:

Claims 1-7 are pending in the present application. Claim 1 is currently amended to by incorporating the limitations of the dependent claim 4. Claim 4 is cancelled. No new matter is added to the claims by the amendments. Accordingly, entry of the amendments is respectfully requested.

Final Rejections under 35 U.S.C. 103(a):

Claims 1, 2, 3, 5 and 6 were rejected under 35 U.S.C. §103(a) as obvious over Grunau et al. (Nucleic Acids Res. (2001) 29:e65, 1-7). Claims 2, 3, 5 and 6 depend from claim 1, and therefore incorporate all the limitations present in that claim.

Rejecting claim 1, the examiner stated that Grunau teaches "incubating the solution comprising the nucleic acid for a time period of 1.5 to 3.5 hours at a temperature between 70 and 90°C, [...], wherein the pH value of the solution is between 5.0 and 6.0." With respect to temperature, the examiner stated that Grunau teaches incubating the solution at 95°C. The claimed range of 70-90°C could therefore be achieved by an ordinary artisan through routine experimentation aimed at optimizing the Grunau method. Similarly, the examiner stated that Grunau teaches final pH adjusted to 5.0. The pH value of the present invention could allegedly be obtained by routine optimization. The examiner cited *In re Peterson*, 65 USPQ2d 1379, 1382 (Fed. Cir. 2003) for the proposition that "a *prima facie* case of obviousness exists when the claim ranges and the prior art range do not overlap but are close enough such that one skilled in the art would have expected them to have the same properties."

In rebuttal, the Applicants point out that first, based on the prior art, pH is not a result-effective variable that is subject to optimization by routine experimentation. Second, Grunau explicitly and implicitly teaches away from the reaction conditions claimed by the Applicants.

The role of pH

Not every parameter may be used to reject a claim for teaching an "obvious optimization by routine experimentation." A parameter in question must be recognized as a "result-effective variable", i.e., a variable which achieves a recognized result, before the determination of the optimum or workable ranges of said variable might be characterized

as routine experimentation. *In re Antonie*, 559 F.2d 618, 195 USPQ 6 (CCPA 1977) (See MPEP 2144.05). Therefore, if the prior art did not recognize the contribution of the variable to the result, optimization of such variable is not obvious.

Grunau does not recognize the role of pH in either stability of the nucleic acid or efficiency and specificity of the cytosine deamination reaction. In contrast, the present invention explicitly emphasizes that changing the pH from 5.0 ("standard conditions") to 5.5 ("optimized conditions") results in a dramatic improvement. The change allows the reaction to proceed quickly and efficiently with no change in specificity at higher temperature without the significant degradation of the nucleic acid. *See* section 1.1.2 "Results" on pp. 14-16, comparing pH 5.0 to pH 5.5.

Furthermore, contrary to examiner's assertions, the range of pH taught by Grunau is significantly different from that of the Applicants' invention. Grunau teaches adding 1200 uL of the bisulfite solution at pH 5.0 to 110 uL of denatured DNA, still in solution of 0.3M NaOH, having pH over 13.0. The inventor followed this protocol and determined that the pH of final solution is near 5.0 (5.02 and 5.05), *see* attached Declaration of the Inventor. Such a low pH value is exactly the "standard conditions" improved upon by the present invention. (*see* pp. 14-16 of the specification and declaration, p. 2, ¶2). Grunau noted that DNA degradation at 95°C was a problem, but did not recognize which parameter needs changing in order to solve the problem. Grunau recognized only the role of temperature, not of pH. ("Raising the temperature up to 95°C can indeed only be recommended only if sufficient amounts of DNA are available because the DNA degrades much faster under these conditions." (p. 5, ¶1)). Therefore pH is not a "result effective variable" as required by MPEP 2144.05. Focusing on the pH and optimizing the pH is not an obvious improvement of the Grunau method. The obviousness rejection is not appropriate for this reason.

The role of temperature

A *prima facie* case of obviousness may also be rebutted by showing that the art, in any material respect, teaches away from the claimed invention. *In re Geisler*, 116 F.3d 1465, 1471, 43 USPQ2d 1362, 1366 (Fed. Cir. 1997). (See MPEP 2144.05). In fact, Grunau explicitly (in the discussion) and implicitly (with data) teaches away from the conditions claimed by the Applicants: "When incubated > 2 hrs at 95°C no PCR product could be amplified anymore. Our data indicate that deamination as well as DNA degradation proceeds faster at higher temperatures giving a time window of 14 h at 55°C but only 1 hour at 95°C." (p. 3, ¶3). Contrary to this warning, the Applicants' improved conditions

allow for incubation for up to 3 hours with optimal results at 2.5 hours (see table in section 1.1.2.1. p. 15). By comparison, using Grunau method, equivalent results are achieved only after 16 hours (see table in section 1.1.2.2. p. 15). Because the Applicants adjusted the pH, degradation was no longer a problem. *See* Declaration, p. 2, ¶2.

As an additional discouragement, Grunau shows that higher temperature results in reduced specificity. The examiner quotes Grunau as stating "However, 5mC is not deaminated at 95°C and as far as this fact is concerned every temperature above 55° can be used." This statement is belied by the data shown in Table 2 of Grunau. With respect to 5mC, both selectivity and specificity of deamination drop by 50% between 55°C and 95°C. (p. 4, Table 2, columns 3, 4). Therefore an ordinary practitioner, carefully reading Grunau, would be discouraged from increasing the temperature for the fear of losing specificity as well as losing the substrate nucleic acid to thermal degradation.

Because Grunau explicitly and implicitly teaches away from using the Applicants' conditions, the obviousness rejection may not be sustained.

Claim 4 was rejected over Grunau in view of Hayatsu et al. (Biochemistry (1970) 9:2858-2865). The limitations of claim 4 are now incorporated into claim 1 and claim 4 is cancelled. Hayatsu uses a pH range of 4-6.5. However, Hayatsu describes deamination of individual nitrogenous bases, not nucleosides, nucleotides or much less, nucleic acid polymers, which are the subject of the Applicants' invention. Therefore the teachings of Hayatsu have no relevance to the issue of thermal stability of polynucleotides during the deamination reaction. A person of ordinary skill seeking to optimize Grunau as it applies to polynucleotides would not have consulted Hayatsu. Therefore an obviousness rejection of claim 4 (or amended claim 1) over Grunau in view of Hayatsu may not be sustained.

Claim 7 was rejected as obvious over Grunau et al. in view of Hayatsu et al. and further in view of Olek et al. (Nucleic Acids Res. (1996) 24:5064-5066). Unlike Hayatsu, Olek teaches deamination of DNA. However, Olek teaches imbedding chromosomal DNA in low-melting point (LMP) agarose blocks. This is done to prevent the loss of DNA during handling when very small tissue samples are used. Olek teaches incubating deamination reactions at 50°C. The use of LMP agarose clearly is incompatible with raising the reaction temperature to 70-90°C. Therefore the teachings of Olek have no relevance to the deamination reaction taking place at higher temperatures. A person of ordinary skill seeking to optimize Grunau by increasing the temperature, would not have

consulted Olek. Therefore an obviousness rejection over Grunau in view of Hayatsu and Olek may not be sustained.

Conclusion:

In view of the above, Applicants believe that all claims now pending in this Application are in condition for allowance. Applicants hereby request continued examination and a three-month extension of time for responding to the final Office Action. The Commissioner is hereby authorized to charge the extension of time fee and the RCE fee (large entity) to Deposit Account No. 50-0812. The Commissioner is further authorized to charge any fee deficiency, or credit any overpayment to the same account.

If the Examiner believes that a telephone conference would expedite prosecution of this application, please call the undersigned directly at 510-814-2706.

Respectfully submitted,

Date: January 16, 2008

O. Kay

Olga Kay (Reg. No. 57,459) 57,459

Roche Molecular Systems, Inc.
1145 Atlantic Avenue
Alameda, CA 94501
Tel: (510) 814-2706
Fax: (510) 814-2973